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Quantity and Quality of Essential Oil of *Pistacia atlantica* Subsp. Kurdica in Response to Gradual Harvest of Oleoresin

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Abstract: Three harvest stages including one-stage (one time after 36 days), two-stage (two times, every 18 days) and three-stage were considered in order to evaluate the effect of gradual harvest of oleoresin on essential oil quantity and quality of male and female *Pistacia atlantica* Subsp. Kurdica. The results demonstrated that essential oil percentage was not affected by genders and treatments. Limonene in male gender and o-Cymene in female gender were more in comparison with each other. Moreover, α-pinene in harvesting of two-stage was lower than other times. Furthermore, 4,8-epoxy-para-mentyh-1-ene in male gender as well as β-cyclocitral in female gender only found in the harvesting of one-stage, while other compounds found in the essential oil were not affected by different stages of gradual harvest.

Key words: Chemical compounds, Essential oil, Gradual harvest, *Pistacia atlantica*.

Introduction

Pistacia atlantica is a dioecious tree belong to Anacardiaceae family [1, 2]. From four sub-species described for *P. atlantica;* three of them including Kurdica, Mutica and Cabulica are grown in Iran [2]. *P. atlantica* Subsp. Kurdica is a native plant of Kurdistan region and it is widely spread around the Kurdish regions of Iran, Iraq, Turkey and Syria [3].

The exploitation from the oleoresin of wild pistachio has a long history in Kurdistan, and it used traditionally for the treatment of peptic ulcer disease and wound healing or to make chewing gum [4, 5]. The oleoresin is containing large amounts of essential oil (EO) as well as α-pinene [6, 3], that can be used for different purposes. Oleoresin is obtained by wounding the trunk and branches of trees in the months of June and July. In order to collect the oleoresin, the clay jars (Kojilla: in Kurdish) were installed below wounds and the harvest period lasts about a one month or more [7]. It is noted that in during the harvest time, the exuded oleoresin is exposed to environmental factors, and it also is susceptible to influence the destructive factors like sunlight, temperature and atmospheric oxygen. Thus, due to the fluidity of oleoresin, exogenous EO and volatility of EOs, it may be decreased the quantity and quality of EO, if lasts the harvest period for a long time. So far, no studies have been done on this issue and the

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available data are limited to postharvest of EOs, that shows some degradation events in EOs when are subject to external factors such as temperature, light, or a combination of both and oxygen [8, 9, 10, 11, 12, 13, 14, 15, 16]. So, the aim of this work was to evaluate the effect of gradual harvest of oleoresin on EO and the compositions of *Pistacia atlantica* Subsp. Kurdica.

Materials and methods

Plant material and treatment setting

The experiment was conducted in the Armerdeh, Baneh, Kurdistan, Iran (35°54'N, 45°47 E; 1645 m above sea level) in Jun - July 2011. For each male and female gender of *P. atlantica* Subsp. Kurdica three trees were selected and trunk of them were injured with hatchet, then the Kojillas (clay jars) were installed below wounds in order to gather the oleoresin.

Three harvest stages including one-stage (one time after 36 days), two-stage (two times, every 18 days) and three-stage (three times every 12 days) were considered as treatments for this study. In each tree, a third of Kojillas were considered for each of the harvest time. The exuded oleoresins were gathered as mentioned in the above and then kept at 4°C until extraction of EO.

Essential oil (EO) extraction

EO of oleoresins was extracted by hydrodistillation using the Clevenger for 2 h, and then expressed as percentage (mL EO per 100 gr oleoresin). The obtained EOs were dehydrated over anhydrous sodium sulfate and stored at 4°C until analysis.

Analysis of EOs and identification of the constituents

GC analyses were carried out by use of Younglin Aceme 6000-GC equipped with a flame ionization detector (FID) and a BP5 capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm). The helium gas was used as carrier gas with flow rate of 0.5 ml/min. The injector and detector temperatures were kept at 290°C with split ratio 1:25 and 300°C, respectively. The oven temperature was programmed to be initially at 50°C for 5 min, then raised to 240°C at a rate of 3°C /min and then to 240°C to 300°C at a rate of 15°C/min and Kept at 300°C for 3 min. The injection volume was 1 μL.

GC-MS analysis was performed using an Agilent 6800-GC coupled with an Agilent 5973 mass equipped with a BPX-5 fused silica capillary column (30 m × 0.25 mm i.d.; film thickness 0.25 μm).The mass spectrometer was used in SCAN mode to detect ions produced by electron ionization at the ion source temperature 220°C and ionization energy 70 eV with scan mass range of 40-500 amu. Oven temperature program, carrier gas and injection volume were the same as mentioned above for the GC.

The components were identified based on their Kovats Index using n-alkanes, and also by comparison with those of the database of the system GC-MS, National Institute of Standard Technology (NIST) library and also by comparison with data from the literature that described by Adams and other published data [17, 18].

Statistical analysis

The experiment was established in a factorial lay out based on completely randomized design with three and two replications for EO percentage and EO composition, respectively. The statistical calculations were performed with SAS software 9.1 [19] and the mean values were compared using the Duncan's test ($p \leq 0.05$).

Results and discussion

Essential oil

As shown in Table 1, the EO content of oleoresin ranged between 28.31 % to 28.89 % and 28.34 % to 28.67 % (v/w) for male and female genders of *P. atlantica* Subsp. Kurdica, respectively. This is in accordance with those obtained in prevising study that was stated 30.80 % and 30.27 % respectively for male and female genders [5]. Nevertheless, it is higher than those reported in oleoresin of *P. atlantica* Subsp. kurdica (20 %) [3] and *P. atlantica* Subsp. mutica (22 %) [6] that collected from Kurdistan in Iran.

Acording to variance analysis the amount of EO was not influenced by gender and mode of gradual harvest of oleoresin (Table 2). Thus the EO obtained from the oleoresin in both male and fe-

Table 1. Chemical composition of the essential oil of *Pistacia atlantica* Subsp. *Kurdica* in response to gradual harvest of oleoresin

Compounds	KI*	Male			Female			Average	
		Three stage	Two stage	One stage	Three stage	Two stage	One stage	Male	Female
Tricyclene	923	0.14 [a]	0.23 [a]	0.16 [a]	0.13 [a]	0.15 [a]	0.12 [a]	0.17 [a]	0.13 [a]
α-Pinene	936	91.58 [a]	89.24 [b]	91.55 [a]	91.69 [a]	91.10 [a]	92.00 [a]	90.79 [a]	91.60 [a]
Camphene	951	0.04	0.12	-	-	-	-	0.05	-
Sabinene	974	0.40 [a]	0.44 [a]	0.41 [a]	0.53 [a]	0.57 [a]	0.55 [a]	0.41 [a]	0.55 [a]
β-Pinene	980	1.95 [a]	2.18 [a]	2.04 [a]	1.88 [a]	1.93 [a]	1.87 [a]	2.05 [a]	1.189 [a]
Myrcene	991	0.40 [a]	0.42 [a]	0.48 [a]	0.40 [a]	0.45 [a]	0.55 [a]	0.43 [a]	0.47 [a]
δ-2-Carene	1001	0.06	0.06	0.07	-	-	-	0.06	-
α-Phellandrene	1010	0.09	0.14	0.10	-	-	-	0.11	-
o-Cymene	1029	0.11 [b]	0.11 [b]	0.08 [b]	0.61 [a]	0.68 [a]	0.68 [a]	0.10 [b]	0.66 [a]
Limonene	1032	0.63 [a]	0.67 [a]	0.65 [a]	0.26 [a]	0.33 [ab]	0.33 [ab]	0.65 [a]	0.31 [b]
β-Phellanderen	1035	-	-	-	0.12	0.15	0.14	0.00	0.14
1,8-Cineole	1036	0.13	0.10	0.12	-	-	-	0.12	-
(Z)-β-ocimene	1039	0.13	0.12	0.14	-	-	-	0.13	-
Terpinolene	1089	0.24 [a]	0.31 [a]	0.38 [a]	0.32 [a]	0.36 [a]	0.20 [a]	0.31 [a]	0.29 [a]
3-Cyclopenthene,1,acetaldehyde, 2,2,3-trimethyl	1106	0.34	0.36	0.28	-	-	-	0.32	-
α-Pinene oxide	1117	0.30	0.43	0.27	-	-	-	0.33	-
trans-2-Caren-4-ol	1136	0.12	0.00	0.03	-	-	-	0.05	-
α-Campholenal	1136	0.04	-	0.06	-	-	-	0.03	-
3-Cyclohexene-1-carboxaldehyde	1150	-	-	-	0.03	0.09	0.06	-	0.06
4,8-epoxy-p-menth-1-ene	1154	0.45	-	-	-	-	-	0.15	-
trans-Verbenol	1154	0.54 [a]	1.38 [a]	0.80 [a]	0.86 [a]	0.81 [a]	0.56 [a]	0.91 [a]	0.74 [a]
p-Cymene-8-ol	1198	0.08 [a]	0.13 [a]	0.10 [a]	0.21 [a]	0.25 [a]	0.26 [a]	0.10 [a]	0.24 [a]
Myrtenol	1205	0.17	0.20	0.18	-	-	-	0.18	-
p-Menth-1-en-8-ol	1205	-	-	-	0.19	0.14	0.11	-	0.14
β-Cyclocitral	1208	-	-	-	0.09	-	-	-	0.03

table 1. (continued).

Compounds	KI*	Male			Female			Average	
		Three stage	Two stage	One stage	Three stage	Two stage	One stage	Male	Female
Verbenone	1219	0.20	0.22	0.12	-	-	-	0.18	-
trans-Carveol	1229	0.14	0.09	0.08	-	-	-	0.10	-
Bornyl acetate	1290	0.34 [a]	0.57 [a]	0.33 [a]	0.31 [a]	0.34 [a]	0.28 [a]	0.41 [a]	0.31 [a]
α-Terpinyl acetate	1353	-	-	-	0.05	0.07	0.07	-	0.06
total compounds	-	98.60	97.50	98.40	97.67	97.41	97.77	98.16	97.61
Essential oil percentage	-	28.89 [a]	28.13 [a]	28.19 [a]	28.67 [a]	28.34 [a]	28.44 [a]	28.40 [a]	28.48 [a]

Means with different letters are significantly different by Duncan's test ($p \leq 0.05$)
* Kovats Index

male genders as well as in all three methods of harvest (one-stage, two-stage and three-stage) was similar and there was no significant difference between them in this respect (Table 1).

Essential oil compositions

The EO composition of male and female *Pistacia atlantica* Subsp. Kurdica analyzed by GC-MS is given in Table 1. Depending on treatment, in male gender about 22 to 24 compounds representing 97.50 % to 98.60 % of the all compounds were identified. Also, the identified compounds in female EO were 15-16 compounds representing 97.41 % to 97.77 % of the total EO. In addition, the chemical analysis of the EOs showed that it was very rich in α-pinene and it was found as the main compound in both male (89.24 % to 91.58 %) and female (91.10 % to 92 %) genders. Moreover, β-Pinene was as another dominant composition in male (1.62 % to 1.64 %) and female (6.85 % to 6.86 %) genders, while other compounds were lower than 1 %.

Similar to our findings, we have previously demonstrated α-pinene (male, 87.66 % to 93.90 %; female, 85.33 % to 92.48 %) and β-pinene (male, 1.57 % to 2.57 %; female, 1.69 % to 2.06 %) as the main compounds in the oleoresin EO of *P. atlantica* Subsp. Kurdica in five investigated populations from the Kurdistan of Iran, with the difference that terpinolen was found as another dominate compound in some populations for both male (0.00 to 4.02 %) and female (0.26 % to 2.40 %) genders [20], but it was lower than 1 % in this study. In the other hand, α-pinene also was determined as the major components in the oleoresin EO of some species of *Pistacia* including *P. atlantica* Subsp. Kurdica [3, 21], *P. atlantica* Subsp. mutica [6], *P. atlantica* [22], *P. lentiscus* var. chia [23], *P. lentiscus*, *P. khinjuk* and *P. terebinthus* [6].

There are some differences between male and female genders in case of minor compositions of oleoresin EO but not in main and dominate compounds. In evaluating compounds with average of the top one percent the results indicated that the compounds including α-phellandrene (0.11 %), 1,8-cineole (0.12 %), Z-β-ocimene (0.13 %), 3-cyclopenthene,1,acetaldehyde,2,2,3-trimethyl

(0.33 %), α-pinene oxide (0.33 %), 4,8-epoxy-para-mentyh-1-ene (0.15 %), myrtenol (0.18 %), verbenone (0.18 %) and *trans*-carveol (0.10 %) only found in male gender. Vice versa, the EO of female gender was containing β-phellanderen (0.14 %) and para-Menth-1-en-8-ol (0.15 %) that were not found in male gender. Also, some fiddling compounds (lower of 1 %) were identified in only one gender (Table 1). In addition, the male and female genders in terms of the content of limonene and o-cymene showed significant differences towards one another (Table 2). The higher amount of limonene were found in the male gender (0.65 %) compared to female gender (0.31 %). On the contrary, the o-cymene was significantly higher in the female gender (0.66 %) than in the males (0.10 %) (Table 1).

This data are entirely consistent with previse studies that indicate there is no chemical variation between male and female genders in terms of main and dominate compounds in oleoresin of *P. atlantica* Subsp. Kurdica; while, the chemical variation only found in case of minor compounds that described in above [20]. Also, in other study [5] have been showed that limonene and sabinene were higher in male gender than in female; whereas, in this study there was no significant difference between the male and female genders in case of sabinene. Nevertheless, this result is contrary with that from in other studies conducted on unripe galls [24] and leaves of *P. atlantica* [25, 26] which indicates chemical diversity between the male and female genders in terms of the main components and chemotypes.

The results of variance analysis in our study demonstrated that α-pinene was affected by gradual harvest of oleoresin (Table 1). The amount of α-pinene was decreased in the harvesting of two-stage compared to other times (one-stage and three-stage) (Fig 1). Moreover, 4,8-epoxy-p-mentyh-1-ene (0.45 %) in male gender as well as β-cyclocitral (0.09 %) in female gender only found in the harvesting of three-stage, whereas, the amounts of other EO compositions were not statistically influenced by gradual harvest (Table 1).

No studies have ever been done in this regard. Our study is the first report that showed the gradual harvest of oleoresin had not remarkable

Table 2. Analysis of variance of chemical composition of essential oil in male and female *Pistacia atlantica* Subsp. Kurdica in response to gradual harvest of oleoresin based on mean squares

CV (%)	Error	Gradual harvest * gender	Gradual harvest	Gender	Sources
-	6	2	2	1	Degree of freedom (df)
0.731	0.444	0.856 ^{ns}	3.172 *	1.968 ^{ns}	α-Pinene
24.818	0.014	0.0001 ^{ns}	0.002 ^{ns}	0.052 ^{ns}	Sabinene
10.765	0.045	0.008 ^{ns}	0.022 ^{ns}	0.078 ^{ns}	β-Pinene
25.219	0.013	0.001 ^{ns}	0.015 ^{ns}	0.004 ^{ns}	Myrcene
13.615	0.003	0.001 ^{ns}	0.003 ^{ns}	0.924 **	ortho-Cymene
30.352	0.021	0.001 ^{ns}	0.003 ^{ns}	0.350 **	Limonene
38.744	0.041	0.012 ^{ns}	0.004 ^{ns}	0.001 ^{ns}	Terpinolene
18.464	0.012	0.006 ^{ns}	0.016 ^{ns}	0.021 ^{ns}	Bornyl acetate
27.253	0.011	0.002 ^{ns}	0.006 ^{ns}	0.005 ^{ns}	Tricyclene
-	5	2	2	1	df
22.792	0.009	0.004 ^{ns}	0.0004 ^{ns}	0.051 ^{ns}	*para*-Cymene-8-ol
21.144	0.039	0.006 ^{ns}	0.051 ^{ns}	0.070 ^{ns}	*trans*-Verbenol
-	12	2	2	1	df
4.471	1.617	1.553 ^{ns}	2.254 ^{ns}	0.032 ^{ns}	Essential oil persentage

** ($P<0.01$), * ($P<0.05$), ns ($P>0.05$)

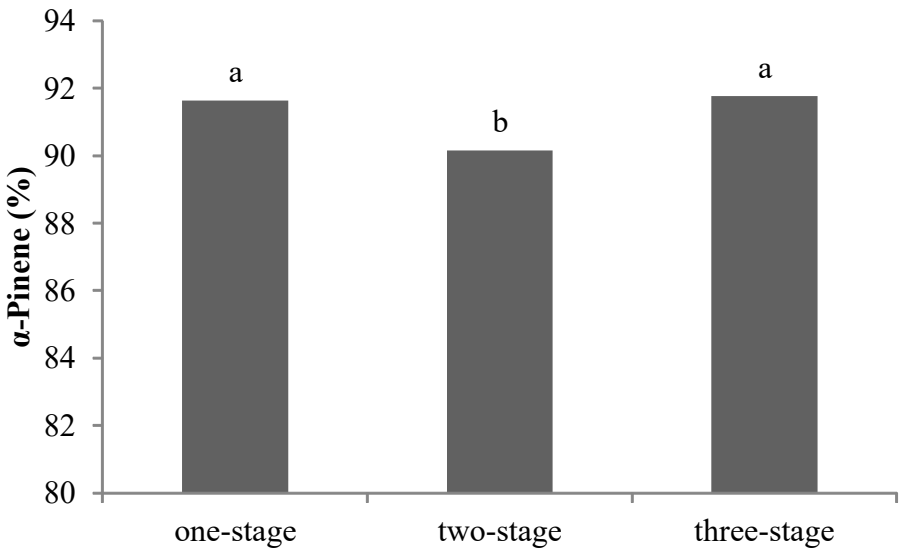

Figure 1. Amount of α-pinene in oleoresin essential oil of *Pistacia atlantica* Subsp. Kurdica in response to gradual harvest of oleoresin

effect on quantity and quality of EO of *P. atlantica* Subsp. Kurdica, except in minor cases that were described in table 1. As it has been previously reported, the EO and its components were not affected by light during the harvest time (36 days), when a part of extracted oleoresin were covered with black tube in order to avoid a light [20]. The available data in associated with our study are limited to postharvest which indicates some change in quality of EOs under the influence of temperature [13, 27], light [15, 28] and oxygen [14, 29].

In conclusion, our results showed that overall the gradual harvest of oleoresin had not remarkable effect on quality and quantity of *P. atlantica* EO. According to our findings it can be derived that there is not sufficient time for the influence the environmental factors such as light, temperature, atmospheric oxygen and other destructive factors during the harvest time that it is common for the harvesting and extraction of oleoresin from the *P. atlantica*. Thus, according to obtained results suggests that the oleoresin be harvested as a single-stage (one time at the end of the harvest period) due to savings in time and costs.

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